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Bone Markers, Calcium Metabolism, and Calcium Kinetics During Extended-Duration Space Flight on the Mir Space Station

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ABSTRACT: Bone loss is a current limitation for long-term space exploration. Bone markers, calcitropic hormones, and calcium kinetics of crew members on space missions of 4–6 months were evaluated. Space-flight-induced bone loss was associated with increased bone resorption and decreased calcium absorption.

Introduction: Bone loss is a significant concern for the health of astronauts on long-duration missions. Defining the time course and mechanism of these changes will aid in developing means to counteract these losses during space flight and will have relevance for other clinical situations that impair weight-bearing activity.
Materials and Methods: We report here results from two studies conducted during the Shuttle-Mir Science Program. Study 1 was an evaluation of bone and calcium biochemical markers of 13 subjects before and after long-duration (4–6 months) space missions. In study 2, stable calcium isotopes were used to evaluate calcium metabolism in six subjects before, during, and after flight. Relationships between measures of bone turnover, biochemical markers, and calcium kinetics were examined.
Results: Pre- and postflight study results confirmed that, after landing, bone resorption was increased, as indicated by increases in urinary calcium ($p < 0.05$) and collagen cross-links (N-telopeptide, pyridinoline, and deoxypyridinoline were all increased >55% above preflight levels, $p < 0.001$). Parathyroid hormone and vitamin D metabolites were unchanged at landing. Biochemical markers of bone formation were unchanged at landing, but 2–3 weeks later, both bone-specific alkaline phosphatase and osteocalcin were significantly ($p < 0.01$) increased above preflight levels. In studies conducted during flight, bone resorption markers were also significantly higher than before flight. The calcium kinetic data also validated that bone resorption was increased during flight compared with preflight values (668 ± 130 versus 427 ± 153 mg/day; $p < 0.001$) and clearly documented that true intestinal calcium absorption was significantly lower during flight compared with preflight values (233 ± 87 versus 460 ± 47 mg/day; $p < 0.01$). Weightlessness had a detrimental effect on the balance in bone turnover such that the daily difference in calcium retention during flight compared with preflight values approached 300 mg/day (–234 ± 102 versus 63 ± 75 mg/day; $p < 0.01$).
Conclusions: These bone marker and calcium kinetic studies indicated that the bone loss that occurs during space flight is a consequence of increased bone resorption and decreased intestinal calcium absorption.
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Key words: weightlessness, calcium absorption, mathematical modeling, stable isotopes, microgravity

INTRODUCTION

Weightlessness-induced bone loss is a major concern for the safety of astronauts on exploration-class missions (≥1000 days).[1–5] Space flight is associated with altered calcium metabolism, including negative calcium balance,[5–7] decreased intestinal calcium absorption,[7,8] increased urinary calcium excretion,[7–10] and subsequent increase in the risk of renal stone formation.[9,10] Although these changes are well documented, we lack an understanding of the time course of these changes and how indirect measures of bone turnover (such as bone markers) relate to more direct measures (obtained from tracer studies). This knowledge is important for proposing and evaluating countermeasures to bone mineral loss. Effective countermeasures that reduce bone loss will not only help ensure the health and safety of crews on shorter missions (such as those used for the International Space Station) but will also contribute to the development of treatments for bone diseases on Earth.[1,11,12]

The authors have no conflict of interest.

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Bone resorption, assessed by measuring urinary concentrations of collagen cross-links[7,13–16] or hydroxyproline,[6,17] is greater during space flight than before flight. Initial calcium tracer kinetic studies conducted on Mir also indicated that bone resorption increases during flight,[7] despite use of exercise countermeasures.

Both the major circulating metabolite of vitamin D (25-hydroxyvitamin D) and the active form of this hormone (1,25-dihydroxyvitamin D) decrease during space flight.[7,18] The depletion of 25-hydroxyvitamin D [25(OH)D] may be related to both dietary insufficiency and lack of UV light exposure during space flight.[1] The decrease in circulating 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] has been reported to occur within 2 weeks after launch, well before changes in vitamin D stores were observed.[7] Serum parathyroid hormone levels also decrease during flight, likely contributing to the observed decrease in both $1,25(OH)_2D$ and calcium absorption.[7,8]

Insight into the mechanisms of flight-induced bone loss comes from the study of factors known to regulate bone and calcium homeostasis and of biochemical markers of bone and calcium metabolism. These factors either modulate or reflect the processes of intestinal calcium absorption, bone remodeling (deposition and resorption), and renal calcium excretion. Calcium stable isotope tracers are useful in elucidating these mechanisms and can be used to measure fractional calcium absorption and rates of bone calcium deposition and resorption.[18,19]

The joint U.S./Russian flights to the Mir Space Station in the mid- to late 1990s afforded a valuable opportunity to study astronauts and cosmonauts during and after long-term space flight. The studies reported here were designed to assess the effects of weightlessness on calcium and bone metabolism. The results of two studies are reported: study 1, an examination of markers of bone and calcium metabolism before and after flight; and study 2, a more comprehensive examination that included in-flight assessment of calcium kinetics and biochemical markers of bone and calcium homeostasis. These studies, in addition to our earlier published preliminary data,[7] provide a unique view of the effects of weightlessness on calcium metabolism.

MATERIALS AND METHODS

Subjects

These studies were reviewed by the Johnson Space Center Institutional Review Board and the Russian Academy of Science Bioethics Review Committee. All subjects provided informed consent before they participated in the studies.

These studies were conducted as part of the Shuttle-Mir Science Program. The subjects included both astronauts and cosmonauts on missions that ranged in length from about 4 to 6 months. Thirteen crew members participated in pre- and postflight studies of biochemical markers of bone and calcium metabolism (designated study 1), and three of them also participated in ground-based and on-orbit studies of calcium kinetics (designated study 2). In the analysis of results from study 2, we also included additional data from three subjects from an earlier publication[7] for comparison and to strengthen the power of statistical analyses. Their space mission was 115 days long. No subjects reported the use of any medication (such as antiresorptives) that would alter bone metabolism.

In general, the U.S. astronauts launched to and returned from the Mir on the Space Shuttle, whereas Russian cosmonauts launched and returned on a Russian Soyuz vehicle.

Study 1 subjects were 12 males and 1 female (mean age, 44 ± 6 years; mean body weight, 78.5 ± 5.7 kg). Study 2 included three of the male subjects (44 ± 3 years; 82.7 ± 5.4 kg) who participated in study 1, as well as previously published data from three additional male subjects (47 ± 12 years; 77.9 ± 7.2 kg).

Study design

Study 1: Blood and urine samples were collected before and after flight for determination of biochemical and endocrine markers of bone and calcium metabolism. Preflight studies were conducted about 6 and again 2 months before launch. Because crew members who later flew to Mir often trained as backup crew members for missions earlier than their own; for many subjects, more than the planned two data collection sessions were completed. For consistency, only the last two blood and urine collections were included in statistical analyses.

Postflight studies with astronauts were initiated at the Kennedy Space Center in Florida within 4 h of landing (return + 0 days [R + 0]), and in Houston on days R + 7 and R + 14. Postflight studies with cosmonauts were performed in Star City, Russia, on days R + 1, R + 7, and R + 14.

Each data collection session included one fasting blood sample and all urine voids collected over a 48-h period. These were processed and analyzed for endocrine and biochemical variables.

Study 2: Calcium kinetic studies were conducted using oral and intravenous calcium stable isotope tracers. Blood and urine samples were collected for determination of stable isotopes and of biochemical markers of bone and calcium homeostasis. Because these samples were collected during flight, as well as before and after flight, these results are presented separately from the study 1 biochemical marker data.

Tracer kinetics protocol: Calcium kinetic studies were performed once during each phase (before, during, and after flight) of each mission. The preflight study was conducted 1–2 months before launch. In-flight studies were conducted after about 60–90 days of weightlessness (the previously published studies were initiated after 110 days of flight). Postflight kinetic studies were planned for 3 days after landing and 6 months after landing.

Except for differences in the specific isotopes and doses used, dual-isotope tracer studies were performed as previously described.[7] Briefly, oral doses of calcium (30 mg Ca, ~98.5% ^{44}Ca enriched; Oak Ridge National Laboratories, Oak Ridge, TN, USA) were delivered in 2 ml of dilute HCl. Intravenous doses (21 mg Ca, ~93.6% ^{42}Ca enriched; Oak Ridge National Laboratories) were delivered in 1 ml of sterile, injectable 0.16 M sodium lactate. Preparation of

these doses has been described previously.[7,20] Tracers were packaged for use in weightlessness, and identical syringes were used for ground and flight studies.

A carrier dose of calcium (250 mg) was ingested immediately after the stable isotope tracer. Calcium carbonate, administered in two capsules, was the carrier (given as 625 mg ± 2% $CaCO_3$, ACS grade, C3049; Sigma Chemical, St Louis, MO, USA). For the pre- and postflight studies, a polyethylene glycol fecal marker was also ingested. This was administered in two gelatin capsules containing a total of 750 mg ± 2% (MW ≈3350; Sigma Chemical).

After subjects received the oral tracer dose (^{44}Ca), blood and saliva samples were taken 1 h later, and the second tracer (^{42}Ca) was injected intravenously. Subjects were not allowed food for 1 h after the intravenous dose. A blood sample was collected about 24 h after the oral dose. Urine, fecal, and saliva samples were collected as scheduled over the following 21 days.

A number of issues arose, during and after space flight, that affected the final kinetic data set. During the first in-flight session, one of the subjects inadvertently ingested the intravenous tracer. During the second in-flight session, another subject took only the oral dose, and blood samples were limited or unavailable for two of the subjects. Although we had hoped to repeat the tracer studies 6 months after landing, two of the three subjects were unavailable, and data are not reported for the one subject who did participate.

Biological sample collection: Blood samples were collected by standard phlebotomy techniques into evacuated blood collection tubes. Except on landing day, baseline blood samples for the kinetic study were collected in the morning after an 8-h fast.

Before and after flight, urine voids were collected into single-void urine containers (Cole-Parmer Instrument, Co., Vernon Hills, IL, USA). Samples were stored with ice packs or refrigerated until they were processed (within 24 h of collection). Aliquots for determination of individual analytes were prepared and stored frozen at −70°C until they were analyzed.

For study 2, saliva, urine, and blood samples were collected during flight as well as before and after flight. Saliva samples were collected onto dried dental-cotton rolls in Salivettes (Sarstedt, Inc., Newton, NC, USA).

In-flight urine voids were collected into urine collection devices containing 1 ml of a LiCl solution, which was used as a volume marker. Samples were mixed, 10-ml syringe aliquots were obtained, and these aliquots were frozen until they were returned to Earth. The in-flight voids of one crew member were preserved using a combination of thymol and thimerosal because freezers were unavailable. Analytical testing was performed to confirm that the preservative did not affect calcium isotope analyses (data not shown). To determine void volume, samples were analyzed for lithium concentration by atomic absorption spectrometry. Twenty-four-hour pools were created from the individual voids for applicable analyses.

Fecal samples (pre- and postflight only) were collected into preweighed individual fecal collection containers (Sage Products, Crystal Lake, IL, USA) and were frozen until analyzed.

Biochemical analyses

Total calcium was measured in serum by spectrophotometric techniques (Beckman CX5 or CX7; Beckman Instruments, Anaheim, CA, USA). Whole blood ionized calcium was determined by using ion-sensitive electrode techniques (i-STAT, Princeton, NJ, USA). The i-STAT instrument used for the ionized calcium measures has been previously verified for use on the ground and during space flight.[21,22]

Total calcium was measured in urine and saliva with a Perkin-Elmer 4000 atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, MA, USA). Fecal total calcium content was determined by flame atomic absorption spectrophotometry with a Smith-Hieftje 4000 instrument (Thermo Jarrell Ash, Franklin, MA, USA).

Serum osteocalcin (Biomedical Technologies, Stoughton, MA, USA) and calcitonin (Nichols Institute, San Juan Capistrano, CA, USA) were measured by radioimmunoassay. Intact parathyroid hormone was determined using a commercially available immunoradiometric assay (Nichols Institute). Both 25(OH)D and $1,25(OH)_2D$ were determined using commercially available kits (DiaSorin, Stillwater, MN, USA). Bone-specific alkaline phosphatase (BSALP) was measured by ELISA (Quidel, Santa Clara, CA, USA). Urinary collagen cross-links (N-telopeptide [NTX], deoxypyridinoline [DPD], and pyridinoline [PYD]) were analyzed with commercially available kits (Quidel; Osteomark ELISA kit; Ostex International, Seattle, WA, USA), as previously described.[7,13,23]

Stable isotope analyses (study 2 only)

Fecal samples were ashed in a muffle furnace and reconstituted in 0.3 N HCl before digestion in concentrated HNO_3 and $HClO_4$. Stable isotopes were analyzed using thermal ionization mass spectrometry techniques, as described previously.[20,24]

Dietary intake (study 2 only)

Each time tracers were administered, dietary intake was determined for 5 days beginning with the day of tracer administration. For preflight and postflight collections, weighed meals were provided to the crew, and any uneaten portion was also weighed. A research dietitian met with the crews before each data collection session to train them to record intake of any additional items. Crew members were also asked to record any medications or dietary supplements taken. After each data collection session, the dietitian met with the crew to review records and verify information. In-flight intake of food and fluids were recorded by means of a barcode reader, which recorded subject identification, time and date of entry, and quantity of each item consumed.

Preflight and postflight dietary intake data were analyzed using the Minnesota Nutrition Data System (NDS) software, developed by the Nutrition Coordinating Center

TABLE 1. BLOOD AND SERUM MARKERS OF BONE AND CALCIUM METABOLISM BEFORE AND AFTER LONG-DURATION SPACE FLIGHT (STUDY 1)

	Preflight	*R + 0*	*R + 1 d*	*R + 5 to R + 10 d*	*R + 11 to R + 17 d*	*R + > 5 mo*
Calcium (mM)	2.41 ± 0.06	2.44 ± 0.09	2.44 ± 0.07	2.37 ± 0.11	2.36 ± 0.06	2.32 ± 0.06*
25(OH)D (mM)	60 ± 18	52 ± 18	58 ± 16	55 ± 13	54 ± 11	77 ± 20
$1,25(OH)_2D$ (pM)	94 ± 28	84 ± 16	128 ± 22	89 ± 26	95 ± 30	96 ± 51
PTH (intact molecule, pg/ml)	31 ± 14	33 ± 9	24 ± 8	33 ± 10	39 ± 9	30 ± 8
PTH (mid-molecule, pg/ml)	147 ± 58	159 ± 18	155 ± 50	158 ± 47	147 ± 43	145 ± 49
Bone-specific alkaline phosphatase	16 ± 5	17 ± 2	20 ± 6	19 ± 5	20 ± 6†	16 ± 4
Alkaline phsophatase	60 ± 13	69 ± 11	59 ± 8	69 ± 14*	70 ± 12*	61 ± 8
Osteocalcin	8.9 ± 3.8	8.6 ± 2.0	11.3 ± 5.2	12.3 ± 5.4	14.7 ± 7.2†	8.6 ± 3.4
Calcitonin	8.4 ± 2.6	7.1 ± 4.4	9.1 ± 2.4	7.6 ± 2.4	8.0 ± 2.9	8.8 ± 3.1
Ionized calcium	1.22 ± 0.04	1.21 ± 0.03	1.22 ± 0.06	1.21 ± 0.03	1.24 ± 0.07	1.20 ± 0.05
pH	7.35 ± 0.04	7.34 ± 0.01	7.37 ± 0.03	7.36 ± 0.03	7.36 ± 0.04	7.35 ± 0.03

Sampling times are shown as time after return (R) from flight (R + X days after landing, where R + 0 is the day of landing).

Thirteen subjects participated in this study; however, because of analytical and individual subject data collection issues, the number of subject data-points varied among analytes and between time-points. See *Statistical analyses* for details of how this was handled.

* Different from preflight mean ($p < 0.05$).

† Different from preflight mean ($p < 0.01$).

(NCC) at the University of Minnesota, Minneapolis, MN, USA (Software Versions 2.7, 2.9, and Nutrient Database 24, 26).[25–27] In-flight intakes were analyzed using the database created from the chemical analysis of space foods performed by the NASA Johnson Space Center Water and Food Analytical Laboratory.

Kinetic modeling (study 2 only)

Data: The enrichment of tracer in serum, saliva, urine, and feces was determined and was converted to percent of the dose administered. These data, as well as calcium intake, serum calcium concentration, and urine and fecal calcium excretion, were used for kinetic modeling.

Model description: The model used to analyze the data were the same as one used previously[7] with the software WinSAAM. Initially the model was used to fit both intravenous and oral tracers for each kinetic study. To identify parameters that differed between studies, kinetic values for each subject were compared across studies. For each subject, the data from all studies were fitted simultaneously. Carryover of calcium tracer was calculated by simulating for the length of the first tracer study, adding tracer to an intravenous and oral dosing compartment, and continuing the simulation. This process was used to calculate carryover for each of the three to five studies for each subject. Initially, the first two studies were fitted, and only the minimum number of parameters necessary and sufficient to fit both studies simultaneously was allowed to differ between the studies. Then the subsequent studies were fitted using the same principle.

Statistical analyses

Study 1: One-way repeated-measures ANOVA was performed with biochemical data to determine if there were significant differences between the postflight and preflight sessions. A priori we chose to test only for differences between these two phases (we did not test for differences among postflight data collection times). Bonferroni post hoc tests were performed to assess specific differences between sessions.

Study 2: Repeated-measures ANOVA was performed to assess whether the within-subject preflight data were similar. Mean preflight data were compared with in-flight and postflight data. Bonferroni post hoc tests were performed to assess specific differences between sessions.

For both studies, significance was assigned to $p < 0.05$. Statistical analyses were performed using SigmaStat (SPSS, Chicago, IL, USA).

Because of analytical (e.g., sample volume limitations) and individual subject data collection issues (e.g., scheduling), the numbers of subject data-points varied among analytes and between time-points. All available data were input for statistical analysis. When data-points are missing, SigmaStat automatically uses a general linear model approach that constructs hypothesis tests using the marginal sums of squares.

RESULTS

Study 1

Results from pre- and postflight blood analyses are shown in Table 1. Blood concentrations of bone formation markers (BSALP, alkaline phosphatase, osteocalcin) tended to be greater after flight than before flight, reaching statistical significance 2–3 weeks after landing. Both 25(OH)D and $1,25(OH)_2D$ concentrations were lower at landing than before flight, and this change approached statistical significance for 25(OH)D ($p < 0.052$).

Data from pre- and postflight urine samples are shown in Table 2. Urine volume (24 h) was significantly lower in the days just after landing than it was before flight, and 24-h creatinine excretion was greater. The decreased volume

TABLE 2. URINARY MARKERS OF BONE AND CALCIUM METABOLISM BEFORE AND AFTER LONG-DURATION SPACE FLIGHT (STUDY 1)

	Preflight	*R + 0*	*R + 1 d*	*R + 2 d*	*R + 5 to R + 9 d*	*R + 10 to R + 16 d*	*R + 17 to R + 30 d*	*R + 1 to R + 4 mo*	*R + > 5 mo*
Calcium (mmol/d)	4.8 ± 1.7	2.7 ± 1.7*	5.4 ± 1.6	5.4 ± 1.9	4.2 ± 1.5	3.5 ± 1.1	3.2 ± 0.1	4.2 ± 2.1	4.0 ± 2.3
Calcium (mmol/mmol creatinine)	0.52 ± 0.44	0.23 ± 0.15†	0.37 ± 0.24	0.44 ± 0.23	0.42 ± 0.33	0.35 ± 0.19	0.34 ± 0.13	0.41 ± 0.22	0.38 ± 0.33
Phosphorus (mmol/d)	28.8 ± 5.5	15.1 ± 11.1‡	18.9 ± 8.3†	19.1 ± 5.1†	28.3 ± 7.1	28.2 ± 6.7	32.2 ± 4.9	26.0 ± 9.8	29.7 ± 6.6
Phosphorus (mmol/mmol creatinine)	3.11 ± 1.94	1.46 ± 1.55‡	1.23 ± 0.70‡	1.54 ± 0.66	2.77 ± 2.10	2.88 ± 1.60	3.36 ± 0.98	2.91 ± 2.36	2.68 ± 1.55
NTX (nmol/mmol creatinine)	36 ± 16	61 ± 12‡	50 ± 15	53 ± 17	45 ± 10	45 ± 17	60 ± 42	39 ± 19	30 ± 9
PYD (nmol/mmol creatinine)	19 ± 6	37 ± 12‡	29 ± 10†	29 ± 9†	30 ± 9‡	30 ± 9‡	34 ± 7*	33 ± 10‡	20 ± 5
DPD (nmol/mmol creatinine)	3.8 ± 1.2	6.9 ± 2.4‡	5.8 ± 2.2‡	5.5 ± 1.2‡	5.8 ± 1.9‡	6.2 ± 1.9‡	5.7 ± 2.0*	6.2 ± 2.9‡	4.1 ± 0.6
Volume (liter/d)	1.66 ± 0.97	0.89 ± 0.55†	0.99 ± 0.45†	1.32 ± 0.66	1.46 ± 0.87	1.43 ± 0.68	1.58 ± 0.88	1.48 ± 0.87	1.39 ± 0.55
Creatinine (mmol/d)	11.5 ± 4.5	18.9 ± 13.2†	18.5 ± 8.7†	14.2 ± 5.7	13.1 ± 5.1	11.7 ± 4.9	10.2 ± 4.5	11.4 ± 4.3	13.1 ± 4.6

Thirteen subjects participated in this study; however, because of analytical and individual subject data collection issues, the number of subject data-points varied among analytes and between time-points. See *Statistical analyses* for details of how this was handled.

* Different from preflight mean ($p < 0.05$).

† Different from preflight mean ($p < 0.01$).

‡ Different from preflight mean ($p < 0.001$).

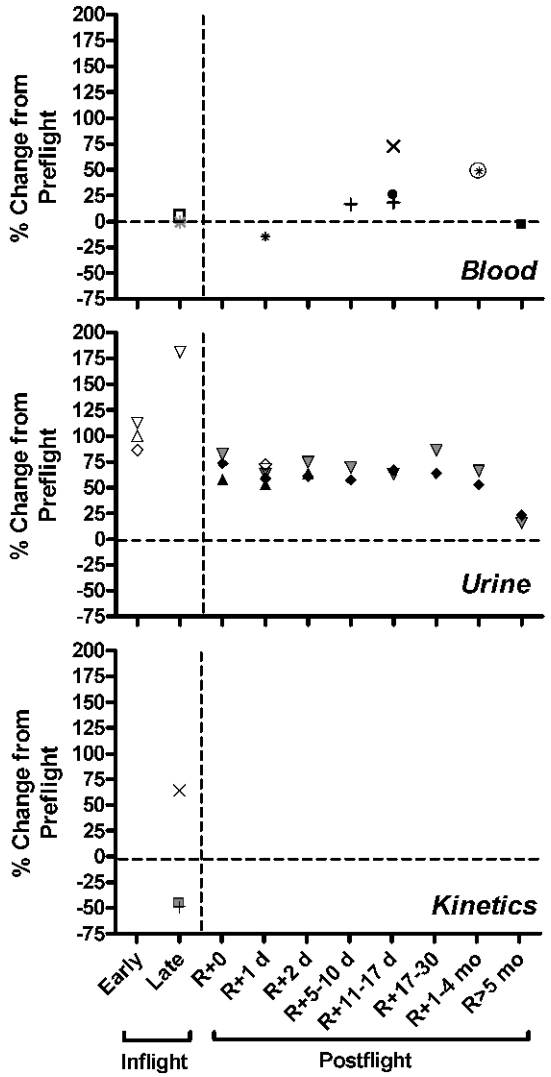

FIG. 1. Summary of biochemical, bone marker, endocrine, and kinetic measures of calcium and bone metabolism. Data are expressed as percent change from preflight for time-points where significant differences were identified. The plots show the timing, degree, and direction of changes, the relationship between the various measures in blood and urine, and parameters of kinetics. (Top) Blood analytes: calcium (■, study 1), bone-specific alkaline phosphatase (●, study 1; ○, study 2), total alkaline phosphatase (+, study 1), osteocalcin (×, study 1), 1,25-dihydroxyvitamin D (*, study 1), ionized calcium (□, study 2), and pH (*, study 2). (Middle) Urine analytes NTX (▲, study 1; △, study 2), PYD (▽, study 1; ▽, study 2), and DPD (◆, study 1; ◊, study 2). (Bottom) Significant calcium kinetic study findings: intake (▣), absorption (+), and resorption (×). The *x*-axis labels match the collection profiles for the blood data; the only difference for urine data was that these were completed at R + 5–9 (instead of 5–10) and R + 10–16 days (instead of 11–17).

was a concern, because the possibility exists that collections may have been incomplete.

Urinary calcium excretion was significantly lower on R + 0 than before flight, and remained lower when the data were normalized to creatinine. Urinary phosphorus was significantly lower on R + 0, R + 1, and R + 2–12. The excretion rate of all collagen cross-links (NTX, PYD, DPD) in

TABLE 3. RESULTS FROM CALCIUM TRACER KINETIC STUDIES BEFORE, DURING, AND AFTER LONG-DURATION SPACE FLIGHT (STUDY 2)

	Subject	*Preflight*	*In-flight 1*	*In-flight 2*	*Postflight*
Calcium intake (mg/d, V_i)	1	1433	481	611	
	2	1063	991	592	
	3	1464	699	676	
	4	1243	503		
	5	1147	400		
	6	950	841		
	Group	1217 ± 204		628 ± 173*	1465 ± 678
Calcium absorption (mg/d, V_a)	1	417	140	178	
	2	499	465	278	
	3	474	226	212	
	4	509	178		
	5	470	164		
	6	389	305		
	Group	460 ± 47		233 ± 87†	439 ± 158
Fractional calcium absorption (%)	1	45	54	43	
	2	78	96	67	
	3	67	52	32	
	4	76	36		
	5	76	77		
	6	69	36		
	Group	69 ± 12		54 ± 21	39 ± 14
Urinary calcium (mg/d, V_u)	1	159	211	150	
	2	432	623	437	
	3	369	234	330	
	4	206	486		
	5	205	219		
	6	204	300		
	Group	263 ± 110		333 ± 143	219 ± 31
Endogenous excretion (mg/d, V_f)	1	178	184	184	
	2	90	93	90	
	3	96	90	90	
	4	114		114	
	5	174		174	
	6	148		148	
	Group	133 ± 39		134 ± 41	170 ± 89
Fecal excretion (mg/d, V_F)	1	1194	525	617	
	2	653	617	403	
	3	1085	561	552	
	4	848	438		
	5	851	410		
	6	709	684		
	Group	890 ± 211		528 ± 99	1195 ± 573
Bone calcium deposition (mg/d, V_{0+})	1	552	587	570	
	2	230	236	229	
	3	698	651	651	
	4	488	166		
	5	521	521		
	6	452	452		
	Group	490 ± 153		434 ± 194	583 ± 141
Bone calcium resorption (mg/d, V_{0-})	1	473	844	725	
	2	254	486	478	
	3	690	749	858	
	4	300	589		
	5	431	751		
	6	416	596		
	Group	427 ± 153		668 ± 130‡	534 ± 125

creased in the days after landing, and PYD and DPD continued to be elevated for several months of the postflight period.

For analytes where postflight data were significantly different from preflight, the postflight data were expressed as percent change from preflight and plotted (Fig. 1). Figure 1

TABLE 3. CONTINUED

	Subject	Preflight	In-flight 1	In-flight 2	Postflight
Bone calcium balance (mg/d, $V_{0+} - V_{0-}$)	1	79	–256	–155	
	2	–24	–250	–249	
	3	8	–98	–207	
	4	188	–423		
	5	90	–230		
	6	36	–144		
	Group	63 ± 75		–234 ± 102†	50 ± 176

Subjects 1–3 completed only one session before flight; for subjects 4–6, the one session closest to flight was used.

Subjects 4–6 are represented by data from a previous publication (7).

Data from the two in-flight sessions are provided where available, but statistical analyses revealed no differences between the sessions, so they were combined for further statistical analysis. To protect subject confidentiality, individual postflight data ($n = 5$) are not shown. The postflight data are from tracer administrations on R + 0 days ($n = 3$) and R + 3 ($n = 2$). Details of other missing data-points are included in the Results section. No data were removed; all of the missing points represent sessions not performed.

* Different from preflight mean ($p < 0.05$).

† Different from preflight mean ($p < 0.01$).

‡ Different from preflight mean ($p < 0.001$).

is intended to highlight the relative changes among all of the findings from this study.

Study 2

Results of the mathematical modeling are shown in Table 3. Fractional absorption was calculated from the tracer data, whereas V_a, the rate of calcium (or tracee) absorption, was determined from fecal calcium excretion and the kinetic data. Because no feces were collected during space flight, it was assumed that, because in-flight fractional absorption did not differ from preflight, tracee absorption did not change, and the preflight values were used (29%, 47%, and 32% for subjects 1, 2, and 3, respectively). Parameters that differed between studies were absorption, fractional urinary excretion, and fraction of serum calcium in saliva. In one postflight study, the fractional transfer coefficient from exchangeable bone back toward extravascular fluid decreased 50%; otherwise the three parameters listed above were necessary and sufficient to fit all data.

Calcium absorption was significantly ($p < 0.01$) less during flight than before flight, and bone resorption was significantly ($p < 0.001$) greater during flight (Table 3). Bone calcium balance was significantly more negative during flight than before flight.

Blood and serum concentrations of biochemical markers from study 2 are shown in Table 4, and urinary excretion rates of the markers are shown in Table 5. Both 25(OH)D and $1,25(OH)_2D$ tended to decrease early in flight (Table 4). By the end of the mission, however, $1,25(OH)_2D$ had returned to preflight levels (Table 4). BSALP and osteocalcin (bone formation markers) followed a pattern similar to that of the active form of vitamin D. The excretion rate of bone resorption markers increased early in flight (Table 5), and PYD and DPD remained elevated well into the recovery period.

When in-flight and postflight data for an analyte were significantly different from preflight data, the in- and postflight data were expressed as percent change from the preflight value and plotted (Fig. 1). Figure 1 is intended to highlight the relative changes among all of the findings from this study.

DISCUSSION

Mitigating the loss of bone during space flight will not only ensure the health and safety of astronauts, but will also enable humans to undertake long-term exploration missions and will have potential Earth applications. The data presented herein show that calcium and bone metabolism were significantly affected by weightlessness and that many of the effects remained, in some cases for an extended period, after space travelers returned to a gravitational environment. Biochemical and kinetic measurements indicated that bone and calcium loss resulted from a combination of reduced intestinal absorption, increased calcium excretion, and increased bone resorption.

The temporal relationships of the results from these studies, as shown in Fig. 1, clearly document the effects of space flight and return to a gravitational field on bone metabolism. Most striking in this figure is the fact that all indices of resorption were 75–125% greater than preflight levels during flight and 50–75% greater after the flight. The increased amounts of formation markers in the weeks after the flight, coupled with the increased resorption, clearly show the metabolic activity leading to recovery of bone throughout the rehabilitation period. The in-flight results for biochemical indices of resorption are also of the same magnitude of change as V_{o-}, the kinetic measure of resorption.

Although they were more limited than the in-flight findings, the pre- and postflight data supported previous studies. Vitamin D status tended to decline (after crew members had spent months without UV light exposure), bone resorption was increased, and bone formation markers were relatively unchanged until weeks after the flight.

Vitamin D increases calcium absorption and affects bone deposition and resorption. The absence of UV light during space flight, along with insufficient dietary sources of vitamin D, likely contribute to astronauts' diminished vitamin D stores on long-term missions.[11] However, despite receiving dietary vitamin D supplements of 500 IU on landing day after the 84-day Skylab 4 mission, astronauts had plasma 25(OH)D concentrations that were slightly less than preflight values.[28] These deficits in 25(OH)D were not

TABLE 4. BLOOD AND SERUM MARKERS OF BONE AND CALCIUM METABOLISM BEFORE, DURING, AND AFTER LONG-DURATION SPACE FLIGHT (STUDY 2)

	Preflight	*Early in-flight*	*Late in-flight 1*	*Late in-flight 2*	*R + 0/1*	*R + 3 to R + 17 d*	*R + > 1 mo*
Calcium (mM)	2.4 ± 0.07	2.35 ± 0.05	2.36 ± 0.09	2.32 ± 0.08	2.41 ± 0.05	2.35 ± 0.07	2.36 ± 0.05
25(OH)D (nM)	57 ± 25	34 ± 9	48 ± 24	36 ± 21	39 ± 22	44 ± 19	67 ± 44
$1,25(OH)_2D$ (pM)	102 ± 34	56 ± 4	90 ± 29	100 ± 41	74 ± 25[§]	95 ± 34	128 ± 54*
PTH (intact molecule, pg/ml)	26 ± 10	16 ± 4	16 ± 8	15 ± 6	25 ± 12	32 ± 17	26 ± 4
PTH (mid-molecule, pg/ml) ($n = 3$)	129 ± 20		123 ± 13	127 ± 15	138 ± 62	181 ± 17	
Bone-specific alkaline phosphatase	11.8 ± 4.1	5.3 ± 2.1	12.2 ± 4.8	9.4 ± 3.0	10.2 ± 2.6	11.2 ± 5.2	15.5 ± 7.5[†]
Alkaline phosphatase	60 ± 12		55 ± 25	57 ± 28		68 ± 22	
Osteocalcin	11.7 ± 4.0	8.3 ± 1.5	10.0 ± 4.3	8.7 ± 2.4	11.5 ± 4.6	16.2 ± 7.0	23.7 ± 6.8[†]
Calcitonin	10.9 ± 9.5	8.2 ± 4.0	8.3 ± 3.2	7.2 ± 2.7	7.9 ± 3.8	8.1 ± 3.7	7.9 ± 4.9
Ionized calcium	1.19 ± 0.05	1.17 ± 0.03	1.26 ± 0.03*	1.22 ± 0.03	1.18 ± 0.01	1.22 ± 0.02	1.20 ± 0.05
pH	7.37 ± 0.01	7.40 ± 0.02	7.34 ± 0.03	7.32 ± 0.01*	7.39 ± 0.05	7.39 ± 0.03	7.37 ± 0.01

Six subjects participated in this study; however, because of analytical and individual subject data collection issues, the number of subject data-points varied among analytes and between time-points. See *Statistical analyses* for details of how this was handled.

Midmolecule PTH data were available for only three subjects and were not available early in flight or after the initial postflight studies.

Early in-flight data were collected after 14 days of flight; late in-flight data were collected after 60–110 days of flight.

* Different from preflight mean ($p < 0.05$).

† Different from preflight mean ($p < 0.01$).

‡ Different from preflight mean ($p < 0.001$).

§ One outlier was removed.

observed on the shorter-duration (28- and 59-day) Skylab missions. It is unknown whether the absorption, half-life, or metabolism of vitamin D, and thus the dietary requirement for it, is altered during space flight.

We confirmed findings that $1,25(OH)_2D$ levels tend to be lower during flight,[11] and we hypothesize that decreases in circulating $1,25(OH)_2D$ are related to decreased parathyroid hormone concentrations, rather than to increased disposal of the vitamin D metabolites.

The changes in endocrine regulation of bone metabolism reflect adaptation to the weightless environment. As the body attempts to adapt to a lower requirement for weight bearing and bone resorption increases, physiologic responses that would be expected include increased serum ionized calcium and subsequent decreases in parathyroid hormone, $1,25(OH)_2D$, and calcium absorption. Obtaining data on the time course of these changes will be important for defining the sequence of primary and secondary effects. These changes, if they continue, will result in a measurable deficit in BMC, as has been noted from DXA measurements performed before and after flight.[2]

The data in this paper that pertain to intestinal absorption and urinary excretion of calcium corroborate the Skylab metabolic balance studies.[5,6,28] The evidence for increased urinary and fecal calcium excretion, presented here and in earlier publications,[5,6,9,28] clearly shows that negative calcium balance occurs during space flight.

We found the net loss of calcium from bone to be about 234 mg/day, very similar to the estimations of calcium loss (200–250 mg/day) from the Skylab balance studies and to estimations of calcium balance at −227 ± 63 mg/day from BMD studies.[29]

Evidence from bone biopsy and histomorphometry suggests that, during bed rest, bone formation decreases.[30,31] However, evidence from measuring biochemical markers and calcium kinetics suggests that bone formation is unchanged or decreased.[7,32–34] This difference likely reflects the anatomic level at which measurements were made: site-specific (biopsy) versus systemic (biochemical markers, calcium kinetics).

In our studies, bone formation, as indexed by BSALP and osteocalcin, was unchanged during space flight but increased after flight. In one subject studied on a Mir flight, osteocalcin decreased.[14] Studies have also shown no changes during bed rest, but increased bone formation after reambulation.[23,32,33] In the experiments reported here, the tracer studies did not detect a change in bone formation (V_{o+}) during (or after) flight. Although our initial hypothesis was that this might have been related to the relatively short duration of these studies,[7] extension of the tracer studies to 3 weeks, as reported here, did not change the conclusion that bone formation is not an important factor in space flight-induced bone loss.

Corroborating the findings from our study, several earlier studies have also found bone resorption to increase during space flight and bed rest.[6,13,17] Urinary hydroxyproline was increased 33% after 84 days of flight,[6,17] and urinary collagen cross-links have been found to increase 150% during space flight.[13] Our calcium kinetic data from this study indicated that bone resorption (V_{o-}) increased about 50%. These consistent results indicate that the changes in bone metabolism involve increased bone resorption.

One limitation of these experiments is the relatively narrow window of mission durations. Examining the changes in bone and calcium homeostasis in the initial days and weeks of space flight, as well as on missions >6 months, is critical to understanding the nature of bone adaptation to weightlessness. To address this limitation, we studied the initial adaptation to space flight on the 16-day Space Shuttle Columbia (STS-107) mission. When the brave and talented crew of Columbia were lost during re-entry on the tragic

TABLE 5. URINARY MARKERS OF BONE AND CALCIUM METABOLISM BEFORE, DURING, AND AFTER LONG-DURATION SPACE FLIGHT (STUDY 2)

	Preflight	*Early in-flight*	*Late in-flight 1*	*Late in-flight 2/3 average*	*R + 0*[§]	*R + 1 d*	*R + 2 d*	*R + 3 to R + 9 d*	*R + 10 to R + 25 d*	*R + 1 to 4 mo*
Calcium (mmol/d)	5.3 ± 1.4	6.6 ± 0.9	7.9 ± 3.8	7.3 ± 2.6	2.7 ± 1.7	6.5 ± 1.4	4.7 ± 1.7	5.3 ± 1.0	4.0 ± 0.7	3.5 ± 0.9
Calcium (mmol/mmol creat)	0.39 ± 0.14	0.63 ± 0.43	0.56 ± 0.35	0.54 ± 0.17	0.20 ± 0.13	0.49 ± 0.23	0.39 ± 0.22	0.49 ± 0.12	0.32 ± 0.12	0.22 ± 0.06
NTX (nmol/mmol creatinine)	29 ± 7	58 ± 40*	63 ± 43*	55 ± 26*	38 ± 9	37 ± 12	39 ± 7	32 ± 10	33 ± 9	27 ± 7
NTX (nmol/d)	473 ± 142	784 ± 408†	695 ± 356	732 ± 293	298 ± 112	641 ± 197	541 ± 212	483 ± 207	469 ± 191	419 ± 193
PYD (nmol/mmol creatinine)	17 ± 2	36 ± 27	44 ± 33*	36 ± 24	29 ± 10	28 ± 10	26 ± 6	23 ± 5	28 ± 3	27 ± 4
PYD (nmol/d)	263 ± 50	487 ± 263*	479 ± 281	469 ± 274*	238 ± 130	484 ± 170*	372 ± 180	342 ± 114	387 ± 101	408 ± 110
DPD (nmol/mmol creatinine)	3.2 ± 0.5	6.2 ± 4.8	7.8 ± 5.5	6.2 ± 4.2	5.2 ± 2.0	5.7 ± 2.9	5.4 ± 1.4	4.6 ± 1.2	5.1 ± 1.1	4.1 ± 0.8
DPD (nmol/d)	50 ± 11	83 ± 48*	86 ± 46	81 ± 50	44 ± 30	95 ± 46†	77 ± 39	66 ± 25	71 ± 21	63 ± 21
Volume (liter/d)	1.20 ± 0.38	1.15 ± .052	0.99 ± 0.28	1.10 ± 0.33	0.61 ± 0.32	1.33 ± 0.70	1.12 ± 0.58	1.41 ± 0.56	1.08 ± 0.27	1.02 ± 0.24
Creatinine (mmol/d)	15 ± 5	15 ± 5	13 ± 4	13 ± 3	15 ± 5	17 ± 12	14 ± 6	11 ± 3	14 ± 5	16 ± 4

Six subjects participated in this study; however, because of analytical and individual subject data collection issues, the number of subject data-points varied among analytes and between time-points. See *Statistical analyses* for details of how this was handled.

Early in-flight data were collected after 14 days of flight; late in-flight data were collected after 60–110 days of flight.

* Different from preflight mean ($p < 0.05$).

† Different from preflight mean ($p < 0.01$).

‡ Because of landing time differences, R + 0 was not assumed to be a complete 24-h pool.

morning of February 1, 2003, in a much smaller matter, the scientific products of this experiment, successfully obtained on orbit, were lost as well.

The data we report here are the result of studies that are very "resource intensive," requiring significant amounts of stowage volume, freezer volume, electrical power for the freezer, and crew time for training and sample/data collection, to name just a few examples. Nonetheless, the information gained from this type of study is critical for understanding and counteracting bone loss during space travel. Assessing bone health after flight can lead to limited conclusions, and the in-flight studies allow a direct assessment during the "treatment" phase. When countermeasures (such as exercise) are evaluated only after the mission, one cannot assess the implications for the countermeasure (or therapy) during the mission, and a linear use and effect of the countermeasure are also assumed. These factors are rarely consistent, and thus limit the conclusions to be drawn from endpoint-only measurements.

Characterization of the mechanism and time course by which bone is lost will increase the ability to develop countermeasures to limit this loss during space flight. Promising ground-based evaluations of exercise and pharmacological countermeasures[23,35,36] suggest that effective countermeasures to space flight-induced bone loss are on the horizon. The study reported here, in which changes in bone markers were measured in the same subjects as bone turnover rates using kinetics, provides a basis for comparing percent change in bone markers to quantitative rate of calcium loss from bone.

Although dietary countermeasures are often considered more pedestrian than exercise and pharmacological countermeasures, they should still be considered to have potential for mitigating bone loss. To provide the building blocks of bone, adequate nutritional support will be required when these other countermeasures are in place and may even provide means to mitigate the loss as well. The obvious countermeasure of adding calcium to the diet may not be effective, but changing the intake of other nutrients, including sodium, protein, and vitamin K, holds promise.[11,37,38] If nutritional intakes are not optimized for maximal retention under microgravity conditions, the observed deficits in bone mineral retention will only be exacerbated.

Solution of this problem of bone loss during space flight will not only help enable human exploration of other planets, but will also hasten advances in treatment of bone diseases of those remaining on this planet.

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